

# Effects of Age on Response of Eggs of Indianmeal Moth and Navel Orangeworm (Lepidoptera: Pyralidae) to Subfreezing Temperatures

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**ABSTRACT** The response of eggs of two pyralid moths to exposure to subfreezing temperatures was studied. Egg age at the time of exposure greatly affected the tolerance of Indianmeal moth, *Plodia interpunctella* (Hübner), and the navel orangeworm, *Amyelois transitella* (Walker), to low temperatures. Indianmeal moth eggs incubated at 28°C for 33 ± 8 h were more tolerant of exposure to temperatures of between -12 and -18°C than eggs 57 ± 8 h old. Additional studies showed that Indianmeal moth eggs between 27 and 51 ± 8 h old and exposed to -15 and -19°C were the most tolerant and that eggs older than 57 ± 8 h were the least tolerant. Navel orangeworm eggs showed a similar response, with eggs between 34 and 74 ± 6 h old being the most tolerant and eggs older than 82 ± 6 h being the least tolerant. Because of their comparative tolerance to low temperatures, middle-aged eggs of both species are most suitable for development for low temperature treatments of between -15 and -19°C.

**KEY WORDS** Insecta, *Plodia interpunctella*, *Amyelois transitella*, cold tolerance

A LARGE PROPORTION of U.S. dried fruits and nuts is produced in California (USDA 1988). A major problem in processing and storage of these commodities is insect infestation, particularly Indianmeal moth, *Plodia interpunctella* (Hübner), and navel orangeworm, *Amyelois transitella* (Walker). Of the two species, only the Indianmeal moth is capable of repeated infestation of the product during storage. Infestations by the navel orangeworm commonly originate in the field and are carried into storage; adults do not reproduce under storage conditions (Simmons & Nelson 1975). Larvae of both insects not only reduce the quality of products by their presence and production of frass and webbing, but they also cause direct damage by feeding.

Current control practices rely on chemical fumigants such as methyl bromide and hydrogen phosphide. Concern over the possible loss of these fumigants through regulatory action or the development of resistance has generated interest in alternative methods. Increased interest in the marketing of organically grown and processed products has also created a demand for methods of insect control other than chemicals. As a result, the use of temperature extremes is being considered for postharvest disinfestation of many commodities. Dried fruits and nuts are especially amenable to these methods because they are more tolerant of

temperature extremes than fresh fruits and vegetables. They are also more valuable per pound than grains, especially after processing.

Because dried fruit and nut packers interested in alternative control methods often have access to commercial freezers, exposure to subzero temperatures is often suggested as a possible alternative treatment. Unfortunately, information on the exposure times needed to control insect infestations adequately is limited (Adler 1960, Mullen & Arbogast 1979). Packers are particularly concerned about survival of the egg stage at freezing temperatures, both because eggs are often the most resistant stage to more conventional treatments and because product contamination by eggs is easy to overlook.

The response of insect eggs to various chemical and physical treatments often changes with age. Eggs of the codling moth, *Cydia pomonella* (L.), were most tolerant of methyl bromide when 1 d old (Gaunce et al. 1980). Tolerance to gamma radiation normally increases with egg age (Brower 1974, Johnson et al. 1990). Moffitt & Burditt (1989) found that codling moth eggs in the red ring stage were the most tolerant of temperatures near 0°C. Consequently, when low-temperature treatments for insect pests of dried fruits and nuts are developed, the effect of egg age on response to subfreezing temperatures must be determined. Our study was done to identify the age of Indianmeal moth and navel orangeworm eggs that was most tolerant of subfreezing temperatures.

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### Materials and Methods

Eggs were obtained from colonies maintained continuously on a bran diet modified from the diet described by Finney & Brinkman (1967) (Tebbets et al. 1978). Indianmeal moth was obtained from a walnut packing house in Modesto, Calif., in November 1967. Navel orangeworm was obtained from the University of California, Berkeley, in October 1966. Adult moths were reared at 28°C, 60% RH, and a photoperiod of 14:10 (L:D).

Indianmeal moth eggs were collected from adults 1–2 d after their emergence. Oviposition jars containing 100–150 adults of both sexes were set up at 1600 hours (PST) and placed in environmental chambers kept at 28°C, 60% RH, and a photoperiod of 14:10 (L:D). Eggs were collected at 0800 hours the next morning. Scales were removed from eggs by gently shaking the collection container under a fume hood. The eggs were then passed through a 32-mesh brass screen to remove clumps of eggs and moth body parts.

Indianmeal moth eggs were treated in small dishes made from hollow plastic test tube caps (13 by 9 mm). The bottoms of the caps were cut off, and 100-mesh brass screen was heat-sealed to the cut edges. An analytical balance was used to weigh out 2.1 mg of eggs ( $\approx 100$  eggs) into each dish.

To obtain navel orangeworm eggs, 25 mating pairs were placed in each of several 0.47-liter paper cups closed with 15-cm filter papers and plastic lids. The centers of the plastic lids were cut out to allow air exchange. The cups were placed with the filter paper ends facing a natural light source. After 24 h, the filter papers were collected and cut into strips with 50 eggs per strip. Because most eggs were laid after sundown, the age of eggs was estimated to be 0–12 h old when first collected.

Subfreezing temperatures were applied with an upright Ultra Low Temperature Freezer (Revco Scientific, Asheville, N.C.). An open, plastic-coated, polyurethane ice chest with inside dimensions of 32.5 by 55.5 by 30 cm was placed on the bottom of the freezer. An enameled pan (27 by 40 by 7 cm) was placed in the ice chest. After the ice chest and pan were allowed to equilibrate to the temperature of the freezer, uncovered plastic Petri dishes containing the eggs to be tested were placed on the pan. Air temperatures in the dishes were recorded with a Polycorder datalogger (Omnicdata International, Logan, Utah) using 36-ga type T thermocouples. Treatment temperatures were reached within 3 min after the Petri dishes were placed in the freezer. Once equilibrium was reached, temperatures remained stable ( $\pm 0.5^\circ\text{C}$ ).

After treatment, eggs were removed to Petri dishes containing about 50 g of bran diet. Strips with navel orangeworm eggs or dishes containing Indianmeal moth eggs were placed on the surface of the diet and held at 28°C, 60% RH, and a photoperiod of 14:10 (L:D) for 7–10 d. The 100-mesh screen in the dishes allowed neonate Indianmeal

moth larvae to move into the diet but retained empty chorions and unhatched eggs. Hatched and unhatched eggs of both species were counted to determine egg hatch.

To determine the temperature and exposure suitable for later studies, we tested the response of two different ages of Indianmeal moth eggs exposed to  $-12^\circ\text{C}$ ,  $-15^\circ\text{C}$ , or  $-18^\circ\text{C}$  for 10, 20, 30, 40, 50, or 60 min. To account for the time needed for eggs to reach treatment temperatures, a cool-down period of 3 min was added to all exposures. Eggs were tested when  $33 \pm 8$  or  $57 \pm 8$  h old. Replicates consisted of a single dish containing 2.1 mg of eggs. Three replicates were used for each temperature and exposure tested, including untreated controls. All three replicates were treated together.

Based on the results from the above test, 25-min exposures to  $-15^\circ\text{C}$  and  $-19^\circ\text{C}$  were used for Indianmeal moth eggs for the remainder of the study. A 3-min cool-down was added to all exposures. Indianmeal moth eggs were first tested when  $9 \pm 8$  h old. For exposure to  $-15^\circ\text{C}$ , eggs were tested every 12 h until they were  $69 \pm 8$  h old. For exposure to  $-19^\circ\text{C}$ , eggs were tested every 6 h until they were  $63 \pm 8$  h old. Three dishes containing 2.1 mg of eggs were used for both treatments and controls at each age tested. The test was replicated three times for a total of  $\approx 900$  eggs per treatment.

Because preliminary studies indicated that response of navel orangeworm eggs to low temperatures was similar to that of Indianmeal moth, 30-min exposures to  $-15^\circ\text{C}$  and 25-min exposures to  $-19^\circ\text{C}$  were selected for treatments. Again, 3 min were added to each exposure for the cool-down. Eggs were exposed to  $-15^\circ\text{C}$  when 12, 36, 60, and  $84 \pm 6$  h old. For exposure to  $-19^\circ\text{C}$ , eggs were first treated when  $18 \pm 6$  h old, then again every 8 h until they were  $90 \pm 6$  h old. Three strips with 50 eggs were used for each age. Strips for the following day's tests were cut out each morning. Three untreated control strips were cut each day. The test was replicated three times for a total of 450 eggs per treatment.

Percentage of eggs hatched was determined 7–10 d after treatment. Abbott's (1925) formula was used to derive corrected mortality for all treatments.

### Results and Discussion

The results from the preliminary test with Indianmeal moth eggs are summarized in Fig. 1. Younger eggs, exposed when  $33 \pm 8$  h old, were the most tolerant. Exposure to  $-12^\circ\text{C}$  for as long as 60 min had little effect on hatch. At the lowest temperature ( $-18^\circ\text{C}$ ), exposures up to 30 min caused little egg mortality. Eggs exposed when  $57 \pm 8$  h old were less tolerant to cold. Egg mortality was  $\approx 40\%$  after exposure to  $-12^\circ\text{C}$  for 50 min, and  $\approx 100\%$  after exposure to  $-18^\circ\text{C}$  for 20 min.

Indianmeal moth eggs  $\leq 45 \pm 8$  h old were tolerant of exposure to  $-15^\circ\text{C}$  for 25 min, with  $\leq 6.6\%$

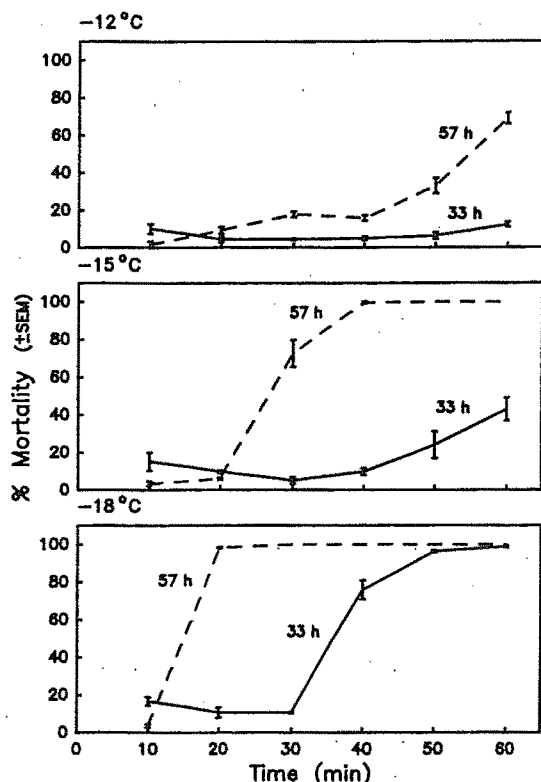


Fig. 1. Mortality of different ages ( $\pm 8$  h) of Indianmeal moth eggs exposed to subfreezing temperatures.

corrected mortality (Fig. 2). Mortality of older eggs increased to  $\geq 83.3\%$ . Differences in tolerance due to age were shown more clearly when eggs were exposed to  $-19^\circ\text{C}$  for 25 min. Mortality of eggs up to  $27 \pm 8$  h old was  $\geq 82.5\%$ . Middle-aged eggs were the most tolerant; mortality dropped to  $\leq 53.6\%$  when eggs were  $27\text{--}45 \pm 8$  h old. For eggs  $>45 \pm 8$  h old, mortality increased with age from 70.2 to 100%. These results show that eggs are most cold-sensitive just before hatch.

Navel orangeworm eggs showed a response similar to that of Indianmeal moth (Fig. 3). Eggs  $\leq 60 \pm 6$  h old were tolerant of exposure to  $-15^\circ\text{C}$  with  $\leq 8.3\%$  corrected mortality. No eggs older than  $60 \pm 6$  h survived the exposure. After exposure to  $-19^\circ\text{C}$ , mortality of eggs  $\leq 34 \pm 6$  h old was high ( $\geq 90\%$ ). Middle-aged eggs ( $34\text{--}74 \pm 6$  h old) were more tolerant; mortality ranged from 45 to 75%. Older eggs ( $>74 \pm 6$  h old) were as cold sensitive as the youngest eggs.

The insects used in our study have been continuously cultured in the laboratory for  $>20$  yr. Because of concern over possible disease contamination, wild strains have not been added to the cultures. Before final recommendations of low temperature treatments can be made, confirmatory tests using recently isolated wild strains should be done.

Previous research has shown that age affects the tolerance of insect eggs to low temperatures. Wat-

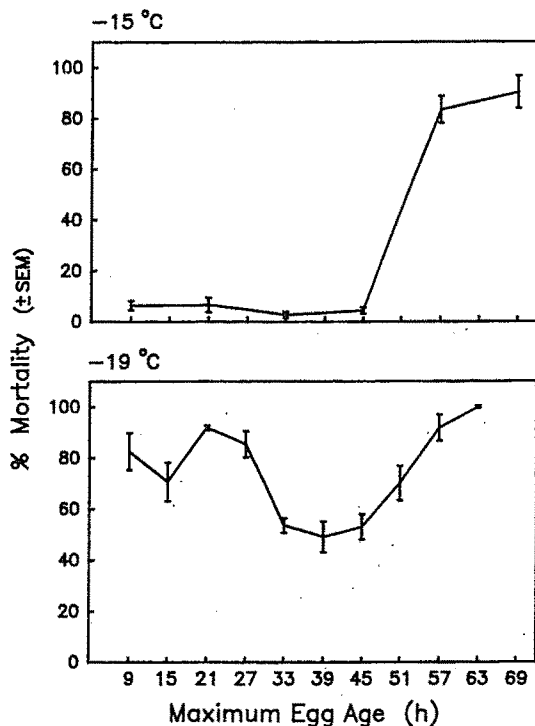


Fig. 2. Mortality of different ages ( $\pm 8$  h) of Indianmeal moth eggs exposed to  $-15$  and  $-19^\circ\text{C}$  for 25 min.

ters (1966) noted that eggs of *Tribolium confusum* Jacquelin du Val  $\leq 4$  h old were less tolerant of exposure to 5 and  $10^\circ\text{C}$  than eggs 1–4 d old. Cline (1970) found susceptibility of Indianmeal moth eggs to  $2.4^\circ\text{C}$  to decrease with age. Similar results were reported by Bell (1975) for Indianmeal moth eggs exposed to 10 and  $15^\circ\text{C}$ . Tebbets et al. (1978) found that the  $LT_{50}$  of 3- to 4-d-old navel orangeworm eggs exposed to  $3.4^\circ\text{C}$  was 4 times greater than that of 0- to 1-d-old eggs. Some research has also documented susceptibility of eggs to low temperatures shortly before hatch. Moffitt & Burditt (1989) found that codling moth eggs in the blackhead stage (96–120 h old) were less tolerant of exposure to  $0^\circ\text{C}$  than younger stages. Kishaba & Henneberry (1966) showed that 2-d-old eggs of *Trichoplusia ni* (Hübner) were less tolerant of exposure to  $10.6^\circ\text{C}$  than 1- or 3-d-old eggs but were more tolerant of exposure to  $4.5^\circ\text{C}$ .

Little information is available on the effect of egg age on susceptibility to subfreezing temperatures. Adler (1960) determined the response of eggs of Indianmeal moth, Angoumois grain moth (*Sitotroga cerealella* (Olivier)) and *Tribolium confusum* Jacquelin du Val to  $-16.7$  and  $3.9^\circ\text{C}$  but did not specify age of the eggs when treated. For Indianmeal moth, a  $LT_{50}$  for  $-16.7^\circ\text{C}$  of 1.6 h was estimated. To determine the response of eggs of the almond moth, *Ephestia cautella* (Walker), to temperatures between 5 and  $-20^\circ\text{C}$ , Mullen & Ar-

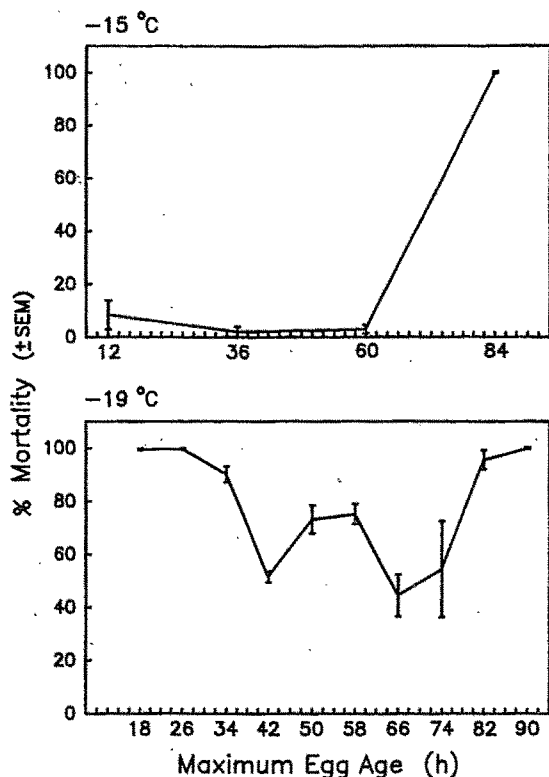


Fig. 3. Mortality of different ages of ( $\pm 6$  h) navel orangeworm eggs exposed to  $-15^{\circ}\text{C}$  for 30 min and  $-19^{\circ}\text{C}$  for 25 min.

bogast (1979) used only 0- to 24-h-old eggs. Because our studies indicate that 1-d-old eggs may be less tolerant than middle-aged eggs to  $-19^{\circ}\text{C}$ , the exposures suggested by Mullen and Arbogast (1979) for control of the almond moth may not be completely effective.

The mechanisms that cause mortality of eggs exposed to temperatures below developmental thresholds are not understood. Howe (1967) suggested that mortality occurs when stored energy reserves are exhausted before development can be completed, or when certain vital chemical reactions are stopped. For temperatures approaching the supercooling point of the eggs, mortality is more likely due to the effects of freezing. Whatever the mechanism, the sensitivity of eggs to low temperatures obviously can vary considerably with age. For this reason, when low-temperature treatments are developed for insect eggs, care must be taken to use the age that is the most tolerant to cold.

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